Application No.: 10/554,374 Docket No.: POLYPROBE 3.3-028

IN THE CLAIMS

(currently amended) A method for producing a sense RNA molecule, comprising:

providing a single stranded cDNA molecule having 5' and 3' ends;

attaching an oligodeoxynucleotide tail to the 3' end of said single stranded cDNA molecule;

providing a double stranded RNA polymerase promoter having a sense strand and antisense strand, wherein the sense strand comprises a single stranded 3' overhang comprising a sequence complementary to said oligodeoxynucleotide tail;

annealing said double stranded RNA polymerase promoter to said oligodeoxynucleotide tail by complementary base pairing with said 3' overhang sequence;

ligating the 5' end of the antisense strand of said double stranded RNA polymerase promoter to the 3' end of said oligodeoxynucleotide tail; and

initiating RNA transcription using an RNA polymerase which recognizes said double stranded promoter, thus producing a sense RNA molecule (sRNA).

- (currently amended) The method of claim 1, wherein 2. a) said attaching comprises providing a mRNA transcript having 5' and 3' ends; and synthesizing a single stranded cDNA molecule from said mRNA transcript.
- (currently amended) The method of claim 2, wherein 3. synthesis of the single stranded cDNA molecule comprises reacting the mRNA moleculetranscript with a RNase H- reverse trancriptase.
- (currently amended) The method of claim 2, wherein single stranded cDNA molecule comprises the reacting the mRNA moleculetranscript with an oligodT primer.

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- (currently amended) The method of claim 2, wherein 5. of the single stranded cDNA molecule comprises svnthesis reacting the mRNA moleculetranscript with a random primer.
- (original) The method of claim 2, further comprising purifying the single stranded cDNA molecule prior to attaching the oligodeoxynucleotide tail.
- (original) The method of claim 6, further comprising 7. degrading the mRNA transcript prior to purifying the single stranded cDNA molecule.
- (original) The method of claim 6, wherein the mRNA 8. transcript is not degraded prior to purifying the single stranded cDNA molecule.
- (original) The method of claim wherein the 9. 1, oligodeoxynucleotide tail is a homopolymeric tail.
- The method of claim 9, wherein the 10. (original) homopolymeric tail is a polydT tail.
- The method of claim 1, wherein the 11. (original) oligodeoxynucleotide tail is attached to the 3' end of the single stranded cDNA molecule using terminal deoxynucleotidyl transferase.
- 12. (original) The method of claim 1 or 2, wherein the double stranded RNA polymerase promoter is a T7, T3, or promoter.
- (original) The method of claim 12, wherein the double 13. stranded RNA polymerase promoter is a T7 promoter.
- 14. (original) The method of claim 1, wherein the single stranded 3' overhang comprises a sequence of adenosine bases.
- (original) The method of claim 1, wherein ligation is performed using T4 DNA ligase.
- wherein RNA 16. (original) The method of claim 1, transcription is initiated using T7 RNA polymerase.

- 17. (original) The method of claim 1, further comprising synthesizing second strand cDNA prior to initiating RNA transcription.
- 18. (original) The method of claim 17, wherein the second strand cDNA is synthesized using DNA polymerase.
- 19. (original) The method of claim 17, wherein the second strand cDNA is synthesized by extension of the 3' overhang of the sense strand of the RNA polymerase promoter.
- 20. (original) The method of claim 17, wherein the second strand cDNA is synthesized using a random primer, thus producing random-primed second strand cDNA fragments.
- 21. (original) The method of claim 20, wherein the randomprimed second strand cDNA fragments are ligated together prior to initiating RNA transcription.
- 22. (original) The method of claim 1, further comprising amplifying the resulting sRNA molecule.
- 23. (original) The method of claim 22, wherein the sRNA amplification is initiated using a combination of oligodT and random primers.
- 24. (original) The method of claim 1, wherein the resulting sRNA molecule comprises a polyA tail.
- 25. (original) The method of claim 24, wherein the polyA tail is attached using polyA polymerase.
- 26. (original) The method of claim 1, further comprising reverse transcribing the resulting sRNA molecule, thereby producing a single stranded cDNA molecule.
- 27. (original) The method of claim 26, wherein the reverse transcription comprises incorporating detectably labeled nucleotides into the single stranded cDNA molecule.
- 28. (original) The method of claim 27, wherein the detectably labeled nucleotides comprise a fluorescent dye.
- 29. (original) The method of claim 28, wherein the fluorescent dye is cy3 or cy5.

- 30. (original) The method of claim 26, further comprising attaching at least one detectable label to the resulting cDNA molecule.
- 31. (currently amended) A method for probing a nucleic acid microarray, comprising contacting a nucleic acid microarray with the detectably labeled cDNA, wherein said detectably labeled cDNA of claim 27, 28, 29, or 30. is prepared by the following steps:

providing a single stranded cDNA molecule having 5' and 3' ends;

attaching an oligodeoxynucleotide tail to the 3' end of said single stranded cDNA molecule;

providing a double stranded RNA polymerase promoter having a sense strand and antisense strand, wherein the sense strand comprises a single stranded 3' overhang comprising a sequence complementary to said oligodeoxynucleotide tail;

annealing said double stranded RNA polymerase promoter to said oligodeoxynucleotide tail by complementary base pairing with said 3' overhang sequence;

ligating the 5' end of the antisense strand of said double stranded RNA polymerase promoter to the 3' end of said oligodeoxynucleotide tail;

initiating RNA transcription using an RNA polymerase which recognizes said double stranded promoter, thus producing a sense RNA molecule (sRNA); and

reverse transcribing a resulting sRNA molecule, thereby producing the single stranded cDNA molecule, wherein the reverse transcribing comprises incorporating detectably labeled nucleotides into the single stranded cDNA molecule.

- 32. (original) The method of claim 2, wherein the mRNA transcript is of mammalian origin.
- 33. (original) The method of claim 2, wherein the mRNA transcript is of human origin.

- (original) The method of claim 2, wherein the mRNA 34. isolated from a biological source comprising transcript is degraded RNA.
- (currently amended) A kit for producing at least one sRNA molecule, comprising: a double stranded RNA polymerase promoter having a sense strand and antisense strand, wherein the sense strand of said double stranded RNA polymerase promoter comprises stranded 3' overhang sequence; single instructional materials for generating sRNA molecules using said double stranded promoter; at least one enzyme for attaching an oligodeoxynucleotide tail onto the 3' end of a single stranded cDNA molecule, wherein the oligodeoxynucleotide tail complementary to the single stranded 3' overhang sequence of said double stranded RNA polymerase promoter; and at least one enzyme for ligating said double stranded promoter onto the 3' end of said cDNA molecule..
 - 36. (cancelled)
- (currently amended) The kit of claim 365, further 37. attaching is comprisingwherein said enzyme for deoxynucleotidyl transferase and wherein said enzyme for ligating is T4 DNA ligase.
- (original) The kit of claim 37, further comprising an oligodT primer; a random primer; dNTPs; and a RNase inhibitor.
- 39. (original) The kit of claim 38, further comprising a DNA polymerase.